AD NUMBER

ADB241196

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies only; Proprietary Info; Sep 98. Other requests shall be referred to USAMRMC, Ft Detrick, MD 21702-5012

AUTHORITY

USAMRMC ltr, 19 Jan 2001

AD_____

GRANT NUMBER DAMD17-96-1-6160

TITLE: Screening for Ataxia-Telangiectasia Mutations in a Population-Based Sample of Women With Early-Onset Breast Cancer

PRINCIPAL INVESTIGATOR: Sharon Teraoka, Ph.D.

CONTRACTING ORGANIZATION: Virginia Mason Research Center
Seattle, WA 98101

REPORT DATE: September 1998

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 1998	Annual (19 Aug 97 - 18 Aug 98)

4. TITLE AND SUBTITLE
Screening for Ataxia-Telangiectasia Mutations in a Population-Based Sample of Women With Early-Onset Breast Cancer

5. FUNDING NUMBERS
DAMD17-96-1-6160

6. AUTHOR(S)
Sharon Teraoka, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Virginia Mason Research Center
Seattle, WA 98101

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution authorized to U.S. Government agencies only (proprietary information, Sep 98). Other requests for this document shall be referred to U.S. Army Medical Research and Materiel Command, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

We are assessing whether a mutated allele of the Ataxia-telangiectasia gene, ATM, is a genetic risk factor for breast cancer, by screening for ATM mutations in patients with early-onset breast cancer. These patients were derived from a large population-based, case-control study of primary breast cancer in Western Washington. We are screening genomic DNAs by SSCP (single strand conformation polymorphism) analysis of the 62 coding exons amplified by multiplex PCR. In 142 early-onset breast cancer cases, we have found no truncation or deletion mutations in the ATM gene. We have detected 6 types of possible missense or splicing mutations and 16 types of rare variants. Most notably, Ser707Pro occurs in 6 cases and only 1 control. The numbers screened to date are still small, and some observed variants may, in fact, be mutations. We plan to increase screening efficiency and expand significantly the number of cases to be screened. Assessment of the prevalence of ATM mutations in breast cancer patients and controls has implications for diagnosis and treatment, given the estimated frequency of AT carriers of 1.4% and their possible hypersensitivity to standard therapeutic doses of radiation.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast Cancer ataxia-telangiectasia, mutation screening, genetic susceptibility, population screening, SSCP	29
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Limited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

____ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

√ AJ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

√ AJ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

√ AJ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Sharon Terada 9/13/98

PI - Signature Date

TABLE OF CONTENTS

SCREENING FOR ATAXIA-TELANGIECTASIA MUTATIONS IN A POPULATION-BASED SAMPLE OF WOMEN WITH EARLY-ONSET BREAST CANCER: ANNUAL REPORT, SEPT. 1998

Sharon N. Teraoka, Kathleen E. Malone, Elaine A. Ostrander, Suna Onengut, Aslihan Tolun, Janet R. Daling, and Patrick Concannon

Virginia Mason Research Center and Fred Hutchinson Cancer Research Center
Seattle, WA 98101, and Bogazici University, Bebek, Istanbul

Introduction

Note that new or revised passages are indicated by borderlines.

Epidemiological studies estimate that 5-10% of all breast cancer cases and 25% of cases diagnosed before age 30 occur in individuals who are genetically predisposed (1). Although mutations in the genes BRCA1 and BRCA2 were once thought to account for 90% of this genetic susceptibility (2), recent studies have detected fewer mutations in these two genes than expected in families with high breast cancer incidence (3,4,5,6) in early-onset breast cancer cases (7,8) and in general populations (9,10). Therefore, other susceptibility genes should be considered. Heterozygosity in the gene for ataxia-telangiectasia (AT), a rare, autosomal recessive disorder, has been suggested as a genetic risk factor for breast cancer (11). AT homozygotes are characterized by progressive cerebellar ataxia and oculocutaneous telangiectases (dilated blood vessels), a 60-180 fold increased incidence of cancer, hypersensitivity to ionizing radiation and radiomimetic chemicals, chromosomal instability, immunodeficiency, elevated levels of serum alpha-fetoprotein, premature aging, developmental defects in various organ systems and a median age of death less than 20 years (reviewed by Gatti (12)). Cultured fibroblasts from AT affected individuals exhibit a characteristic phenotype. AT cells have decreased viability after exposure to ionizing radiation and exhibit radioresistant DNA synthesis (RDS), an inability to shut down DNA synthesis after x-ray exposure, suggesting defects in cell cycle control.

Although AT is rare, AT carriers are thought to be relatively common, estimated in epidemiological studies to be as much as 1.4% of the general population (13) and from a control population in a mutation screening study to be 1% of the general population (14). AT carriers display a heightened sensitivity to ionizing radiation intermediate to that of AT homozygotes and controls (15,16), suggesting a possible link between radiation exposure and genetic susceptibility to breast cancer mediated by the AT gene. Data from several epidemiological studies mentioned in the original proposal (11,13,17,18,19) were pooled, showing that AT heterozyotes have an

increased risk for breast cancer of 3.9 (20). More recently, a study of cancer incidence in 750 blood relatives in 99 AT families confirmed a statistically significant increase in breast cancer, with an estimated risk of 3.8, among AT carriers identified by genotyping with dinucleotide repeat markers in and around the AT gene (21).

The AT gene, ATM, was discovered in 1995 (22,23) and is a member of the phosphatidylinositol-3 kinase (PI-3 kinase) family by virtue of significant sequence homology in the carboxy-terminal tenth of the protein (~350 amino acids) to the catalytic domain of PI-3 kinases. ATM, therefore, plays a role in detection of DNA damage and control of cell cycle progression. Interestingly, BRCA1 and BRCA2 have now been shown to be involved in the response to DNA damage (reviewed by Marx (24)).

The cloning and identification of the AT gene enables us to test directly the hypothesis that a mutated AT allele is a genetic risk factor for breast cancer, by screening for ATM mutations in patients with early-onset breast cancer. We are screening for ATM mutations in patients derived from a large population-based, case-control study of primary breast cancer diagnosed from 1983-90 in white women born after 1944 who were residents of three counties in Western Washington at the time of diagnosis. This case/control study was conducted by the Epidemiology Program at the Fred Hutchinson Cancer Research Center.

We delineated the exon/intron structure of the 5.9 kb cDNA portion of ATM published initially (22) and synthesized primers to amplify each of the exons. Dr. Yosef Shiloh generously provided us with the then unpublished sequences of the intron/exon boundaries for the remaining 5' half of the ATM cDNA (~10 kb full length), enabling us to design primers to amplify the remaining exons and obviating the need for task 1 of the first technical objective in the Statement of Work. The genomic organization of ATM has now been published, describing the large, 146 kilobase gene consisting of 66 exons (25, 26). Many laboratories have contributed to a growing list of AT mutations. The 115 AT mutations that have been published were reviewed by Concannon and Gatti in 1997(27). Since 68% of these mutations are unique and are distributed across the length of the coding region, any study screening for mutations in ATM must screen all exons. Gilad et al. reported that 89% of the AT mutations they identified are null mutations, expected to inactivate the ATM protein by truncation, incorrect initiation or termination, or large deletion (28). However, from the published list of mutations only one mutation has been identified for many of the AT compound heterozygotes, suggesting that missense mutations that are more difficult to detect may exist. In addition, some of the earlier reports of AT mutations catalogued many exon skipping mutations, but did not describe the underlying genomic mutation. Some of the the skipped exons may be due to alternative splicing that occurs in normal controls as well as AT patients. Our study analyzes intron/exon boundaries for possible splicing mutations and attempts to detect single nucleotide variations.

Since 1997, many additional ATM mutations have been published and logged into ATM mutation database websites http://www.vmmc.org/vmrc/atmpat.txt and http://www.uwcm.ac.uk/uwcm/mg/hgmd0.html. Seventeen missense mutations are listed in the former as of Sept. 9, 1998. Furthermore, missense mutations were more prevalent than truncation mutations among ATM mutations found in T cell leukemia (T-PLL) patients (29, 30) and ATM missense mutations were found in British AT patients who developed breast cancer, leukemia, or lymphoma (31). These data underscore the need to screen for single nucleotide changes in ATM in breast cancer patients.

We are screening for ATM mutations in the early-onset breast cancer genomic DNAs by SSCP (single strand conformation polymorphism) analysis of the 62 coding exons amplified by PCR (polymerase chain reaction). We have increased the efficiency of screening by devising multiplex PCR reactions combining up to four exons at a time and running up to 8 exons on a single SSCP gel. Two different gel conditions, MDE and MDE with 5% glycerol, were used to improve detection of single nucleotide differences. Variants detected by SSCP were confirmed by automated sequencing. Variants were checked against age-matched controls to assess the likelihood that the variants are mutations rather than polymorphisms.

We proposed initially to screen 80 early-onset breast cancer cases and an equivalent number of age-matched controls for which DNA was already purified and screened for BRCA1 mutations (7). We are expanding the study as more samples become available. We completed screening the genomic DNA of 87 early-onset breast cancer cases in the first year and an additional 55 in the second year and have found no null mutations in the ATM gene. However, we have detected 5 types of putative missense mutations in exons 8, 15, 30, 62, and 65 and one possible splice mutation in intron 54. Currently, there is no functional assay available to assess the significance of these single amino acid substitutions. However, two of the substitutions occur in regions of the ATM gene of possible functional significance. In addition, the putative mutation in exon 15 corresponds to one of three amino acids altered in an AT patient. We have detected this putative missense mutation in 6 breast cancer patients and in only one control. We have also detected common polymorphisms or rare variants described previously in a studies of Swedish breast cancer patients (32, 33) and rare variants not previously described, some of which may be mutations.

ATM heterozygosity at particular sites, such as exon 15, 2119, appear to play a role in early-onset breast cancer. However, the numbers screened to date are still relatively small, and further screening is necessary to establish the statistical significance of the observed ATM variants. If we can increase screening efficiency, we will continue to expand the numbers of breast cancer cases and controls to be screened. Assessment of the prevalence of ATM mutations in breast cancer patients and controls has implications for increasing our understanding of the diagnosis and treatment of breast cancer, given the

estimated frequency of AT carriers and their possible hypersensitivity to standard therapeutic doses of radiation.

Materials and Methods

Breast Cancer Patient Population. Dr. Janet Daling, Kathleen Malone and colleagues of the Epidemiology Program at the Fred Hutchinson Cancer Research Center have collected blood samples and conducted detailed interviews for a large population-based study of early-onset breast cancer. As a result of previous and current studies, they have recruited a cohort of over 1400 women with breast cancer as well as an equivalent panel of matched controls. The cohort includes cases of primary invasive breast cancer diagnosed in a 3 county area of Washington between Jan.1, 1983 and Apr. 30, 1990 among women born after 1944 and additional cases diagnosed between May 1, 1990 and Dec. 31, 1992 among women under the age of 45. Controls were randomly ascertained and frequency age-matched. Structured interviews of the individuals provided information on reproductive history, contraceptive practices, lifestyle, medical history, basic demographic information, and family history of cancer. Blood samples were collected from 811 cases and blood samples from a comparable number of controls are currently being collected. For our initial studies, Dr. Elaine Ostrander has provided the DNA she has extracted from samples of early-onset breast cancer patients (diagnosed under the age of 35) enrolled in Dr. Daling's study.

Single Strand Conformation Polymorphism (SSCP) Analysis. ATM exons were amplified from 50 ng genomic DNA of each of the study subjects. Oligonucleotide primers used to amplify the exons, including ~50 nucleotides of intron sequences flanking the exons on both sides, are listed in Table 1. Any exons containing variants were given priority when screening control individuals. PCR reactions contained 200 micromolar dCTP, dGTP, dTTP, 20 micromolar dATP, 3 micromolar each primer, .5 microcuries ^{33}P-dATP (Amersham), 1.25 units Hot Tub polymerase (Amersham), Amplitaq (Perkin-Elmer) or Taq DNA polymerase (Boehringer Mannheim), and 1X corresponding reaction buffer in a total volume of 50 microliters. Initially, exons were amplified one at a time, but in order to increase throughput, most exons were combined in PCR reactions amplifying 3 or 4 exons at once. In these multiplex PCR reactions, primer concentration was reduced as much as one half per primer and ^{33}P-dATP increased to .1 microcuries, but all other ingredients and volumes were kept identical to the single exon reactions. SSCP analysis was modified from Orita et al. (34). After denaturing 5 min. at 94°C, PCR was carried out for 35 cycles of 94°C, 30 s, 54°C, 30 s, 72° C, 1 min. For high throughput, some PCR reactions were carried out in V bottom microtiter dishes in a twin block Ericomp plate PCR machine. Ericomp tube PCR machines and MJ Research PCR machines were

This page contains proprietary data.

also utilized. Samples were denatured and separated on MDE (FMC) gels with or without 10% or 5% glycerol.

Direct Sequencing of PCR Products. Highly specific (single band), high yield PCR products were purified on QIAquik Spin PCR Purification kit (Qiagen) or High Pure PCR Product Purification kit (Boehringer Mannheim). 5-10 microliters of PCR product served as template in reactions using the Taq DyeDeoxy terminator cycle sequencing kit (ABI). Unincorporated DyeDeoxy terminators were removed using Centri-sep columns (Princeton Separations). Sequencing reactions were analyzed on an ABI 373A sequencer.

Results

One hundred forty two early-onset breast cancer cases collected in western Washington were screened ~30 at a time for mutations in the 62 coding exons of ATM. Eighty two age-matched controls were also screened. For most of the exons that contained variants, additional controls were screened so that a comparable number of cases and controls were screened. Nucleotide numbering is based on the sequence reported by Savitsky et al. (23) with position 1 representing the first nucleotide of the start codon, and variants are named in accordance with the convention of Beaudet and Tsui (35), modified by Antonarakis (http://ariel.ucs.unimelb.edu.au:80/~cotton/antonara.htm). Exons are numbered in accordance with Uziel et al. (26). The results of the SSCP analysis are listed in Table 2 and an example of a multiplex SSCP analysis is shown in Figure 1. No truncation or deletion mutations were detected. However, at least 5 types of putative missense mutations and one putative splicing mutation were discovered in the breast cancer cases.

Six breast cancer patients have a T>C change at position 2119 of exon 15, leading to a serine to proline change at codon 707. Figure 2 shows the SSCP pattern of the variant band in three of the breast cancer cases. This non-conservative substitution is also part of a mutation, 705tyrosine>phenylalanine, 706serine>isoleucine, 707serine>proline, found in a Swedish AT patient (33). The 2119T>C variant occurred only once in 142 controls screened.

Two breast cancer patients have single amino acid substitutions in regions of ATM of possible functional significance. Figure 3 depicts the 4158C>T nucleotide substitution in patient 2-11 that codes for a serine to leucine change at codon 1383, just after a proline-rich motif recently identified as a SH3 binding site (36). ATM has been shown to interact with c-abl oncogene, which has an SH3 domain, and to kinase c-abl in fibroblasts that have been irradiated (36,37). Patient 2-16 has an arginine to glycine change at

codon 2912, in a highly conserved segment of the PI-3 kinase domain. Neither of these variants has been seen in 111 controls.

Other putative missense mutations occur in regions of ATM where the function is less well understood; exon 8, 609C>A, which changes aspartic acid to glutamic acid, and exon 65, 9031A>G, which changes methionine to valine (Figure 4). Neither of these variants has been seen in 113 controls.

The putative splicing mutation IVS54+8A>G, creates a potential new splice donor site 6 nucleotides after the canonical GT splice donor.

Another non-conservative amino acid substitution was found in two breast cancer cases: aspartic acid to valine at codon 1853 due to a single base change, 5558A>T. However, the substitution also occurs in 3 out of 183 controls. We have also detected a common polymorphism previously described by others (33,38) at the neighboring nucleotide 5557 where a single base change from G to A leads to codon 1853 changing from aspartic acid to asparagine. In our study population there was no significant difference between the frequencies of the two alleles in breast cancer cases vs. controls. The frequencies were .12/.88, A/G in breast cancer cases (158 chromosomes screened) and .15/.85, A/G in controls (236 chromosomes screened). However, a preliminary report from Izatt et al. (38) finds 36% of a small sample (25 patients) of early onset breast cancer cases (<40 at diagnosis), including some who suffered late radiotherapy reactions have the 1853 aspartic acid to asparagine substitution. At this time it is unclear whether either substitution at codon 1853 plays a role in breast cancer susceptibility. In fact we found one breast cancer case with both an asparagine allele and valine allele at codon 1853. It is unclear whether the absence of aspartic acid at codon 1853 is significant.

We detected several rare variants of ATM among our panel of early-onset breast cancer cases. Some of these were reported earlier by others (33), including exon 19 2572T>C (858F>L) and exon 36 5071A>C (1691S>R). These variants are unlikely to be mutations because they were found in controls at similar frequencies. On the other hand, 5071A>C has also been reported as a mutation (31). One notable intron variant is IVS16+22A>C, +34A>C, with substitutions at two different nucleotide positions (Figure 4D). Though far from the consensus splice site, it occurs in two breast cancer cases with a family history of breast cancer and has not been seen in controls. Other rare variants listed in Table 2 lead to conservative substitutions or do not result in amino acid substitutions at all. Four other rare variants found in intron sequences are unlikely to be mutations because they are outside of known splicing consensus sites. However, in light of recent studies suggesting sequence variation in introns and exons distant from known splice consensus sequences can greatly influence correct splicing (39), and preliminary evidence of ATM missense mutations involved in T-cell prolymphocytic leukemia (29,

30), the rare variants found in breast cancer patients cannot be ruled out as mutations.

During the course of our SSCP analysis, we also came across common polymorphisms in ATM exons (or introns) 4, 12, 18, 23, 26, and the aforementioned exon 39. Analysis of exon 39 by SSCP is complicated by the presence of 3 different rare variants in the neighboring intron 38 (data not shown).

Most of the ATM variants listed in Table 2 are single nucleotide differences. We found that adding 5% glycerol to the MDE gel matrix greatly improves resolution of single nucleotide changes in most cases and that 5% glycerol resolves as well as 10% glycerol with the advantage of slightly faster gel running time and ease of handling. In many cases, the variant band is detected in the glycerol MDE gel, but not the non-glycerol MDE gel. However, we believe the non-glycerol MDE gel is still necessary, since a few of the variants are better resolved in the non-glycerol gel and often, the greater separation between the variant and wild type bands allows cleaner physical isolation of the variant band from the gel for subsequent sequence analysis. Figure 1 shows one variant detected more easily in 5% glycerol MDE and another variant detected more easily in non-glycerol MDE.

Because of the large scale of the ATM screening project, we attempted to combine primers for several different exons in the same PCR amplification reaction. For ATM, although the relative uniformity of exon size from ~200-350 bp is optimal for SSCP variant detection, the similarity of size is a disadvantage for multiplexing. In practice, 4 exons at once was the upper limit. Table 3 lists many groups of ATM exons that are compatible for multiplex SSCP analysis.

Discussion

We have accomplished technical objectives 1-4 of the Statement of Work of our research proposal, originally projected to take up to 18 months. One major factor expediting our work was that technical objective 1, task 1, determining the exon/intron structure of the 5' portion of ATM, became unnecesary when Dr. Yossi Shiloh provided that information prior to publication. In addition, technical improvements such as multiplexing of SSCP reactions sped the screening process, allowing us to finish technical objectives 3-4; screening for ATM variants in 80 early-onset breast cancer patients and verifying any SSCP variants by direct sequencing of PCR products. We have completed technical objective 5, task 1, screening for specific ATM variants in 80 controls. Studies of ATM protein function in this and other laboratories has been hampered by difficulty in reproducibly expressing the large and unstable protein in cell culture. Therefore we are continuing to work on task 2, assessing the functional significance of some of the putative missense mutations we have discovered in the breast cancer cases, originally projected for completion by 24 months.

We have not begun task 3, comparing case histories of AT carriers and controls for prior exposure to radiation. With our collaborators, we have decided to wait until the screening is completed to reveal the case histories of the cases and controls so as not to bias SSCP data interpretation. Further, DNA samples to be screened in the future will not be identified in terms of disease status until after screening for ATM mutations is completed.

In the next year we will extend our analysis to an additional 245 breast cancer patients. Although we originally proposed to screen in control DNAs only those ATM exons in which variants had been discovered in breast cancer cases, increases in the efficiency of our screening procedure will now allow us to screen all the exons in order to establish the frequency of AT carriers in this population. We will attempt to screen comparable numbers of cases and controls.

Many other laboratories have undertaken studies similar to ours screening breast cancer patients for ATM mutations. Some of these focus on early-onset breast cancer cases or breast cancer patients from families with high cancer incidence, and others on breast cancer patients with prior exposure to ionizing radiation or hypersensitivity to radiation therapy. Most of the published reports found no evidence of definitive ATM mutations in the breast cancer cases or found no significant increase in ATM mutations compared to controls. Vorechovsky et al. first analyzed 38 breast tumors for ATM mutations by SSCP of both cDNA and genomic DNA and found no mutations (32). Next, genomic DNA from 88 breast cancer patients from Swedish cancer prone families was analyzed by SSCP and 3 ATM mutations were identified (33). However, the AT alleles did not cosegregate with tumors in these cancer families. As the authors mention, although this doesn't rule out ATM as a breast cancer susceptibility gene, larger studies are required to establish statistical evidence.

The largest study we are aware of asking whether ATM heterozygosity is a genetic risk factor for early-onset breast cancer was published by Fitzgerald et al. (14). Analysis of 400 breast cancer patients and 200 age-matched controls from the Boston area showed no evidence that ATM heterozygosity confers predisposition to early-onset breast cancer. However, in an accompanying editorial, Bishop and Hopper contend that the number of individuals tested is still not large enough to exclude a role for ATM in breast cancer susceptibility (40). They applaud Fitzgerald et al.'s study as well as Athma et al.'s study showing AT carriers in AT families are at increased risk of breast cancer (21) and note that conclusions will eventually become clear from identifying consistencies from a number of studies or from large-scale population-based studies. Our study will contribute toward this pool of data.

Furthermore, Fitzgerald et al. utilized the protein truncation assay (PTT) based on in vitro reverse transcription of mRNA coupled with translation, assuming that 90% of ATM mutations in AT patients are chain terminating mutations. Protein truncation mutants may be overestimated due to a combination of greater difficulty in detecting missense mutations and the

probability that some exon skipping mutations for which the genomic mutations were not reported may be alternative splice products rather than actual mutant transcripts. Further, estimates of 80-90% truncation mutations (21) are calculated from samples drawn from multiple populations some of which are inbred or show founder effects. A better comparison for our study in the U.S. would be a recent survey of British AT patients that found only 71% of mutations were truncations (31).

Also, it is unknown whether ATM mutations that might play a role in breast cancer susceptibility would be the same type of mutations that lead to AT. Preliminary evidence from analysis of tumor DNA from patients with sporadic T cell prolymphocytic leukemia (T-PLL), shows a high frequency of mostly missense ATM mutations clustered in the PI-3 kinase domain (29). ATM missense mutations were found in British AT patients who developed breast cancer, leukemia, or lymphoma (31). Our preliminary results indicate ATM missense mutations may be involved in early-onset breast cancer in a Western Washington population.

Although we have detected no null mutations in our early-onset breast cancer population, five putative missense mutations and other rare variants require further investigation. The identification of missense mutations in the PI-3 kinase domain of ATM in T-PLL patients and the report of an AT patient with a missense mutation, 8711A>G, 2904Glu>Gly in the kinase domain (28) support the possibility that the ATM variant we detected in breast cancer patient 2-16, 8734A>G, 2912 Arg>Gly, is a mutation that disables the PI-3 kinase function of ATM. Currently c-abl oncogene is the only known target of the ATM kinase. The ATM variant 4158C>T, 1383 Ser>Leu in breast cancer patient 2-11 might affect the binding of c-abl to ATM since codon 1383 follows the 10 amino acid SH3 binding motif shown to bind in vivo and in vitro to c-abl (36). Baskaran and colleagues demonstrated that mouse embryonic fibroblasts derived from the ATM knockout mouse are defective for radiation induced activation of c-abl (37). If the ATM variant in patient 2-11 disrupts binding to c-abl and radiation induced activation of c-abl is disrupted, the cellular response to DNA damage may be affected.

Conclusions

One of ATM's functions appears to be a response to cellular damage caused by ionizing radiation. Exposure to occupational and fluoroscopic diagnostic radiation radiation and exposure to large doses of radiation such as in atomic bomb survivors and women with repeated fluoroscopy are risk factors for breast cancer (17, 41, 42). The radiosensitivity of AT heterozygotes could put them at risk from standard diagnostic or occupational radiation exposures.

The ATM gene possibly initiates the pathway through which cells deal with the environmental risk for breast cancer, ionizing radiation.

Recent evidence suggests that BRCA1 and 2 participate in the cellular response to DNA damage via radiation as does ATM, giving us further reason to consider ATM as a third breast cancer susceptibility gene. BRCA1 has been shown to bind to the known repair protein RAD51 and both co-localize to nuclear foci in mitotic and meiotic cells (43). ATM, BRCA1, and RAD51 bind to synaptonemal complexes of chromosomes during meiosis; BRCA1 and RAD 51 binding predominantly to asynapsed portions of chromosomes (43) while ATM binds to synapsed portions (45). BRCA2 was also shown to bind to RAD51(45). Furthermore, although mice with inactivated BRCA2 are arrested at day 6.5 of development, Sharan et al. harvested trophoblast cells from these mice and showed they were extremely radiation sensitive (45). Three groups reported the consequences of targeted disruption of ATM in mice, generally, a recapitulation of the AT phenotype, except that ataxia was not pronounced (46, 47, 48). Mice with BRCA1or 2 disrupted were arrested early in embryonic development (49, 45, 50). However, some mice with mutated BRCA2 survived and had phenotypes which greatly resembled those of mice with ATM inactivated(50). The similarities in function and localization suggest BRCA1, BRCA2 and ATM may be involved in the same pathways of the response to DNA damage, albeit at different steps.

Fitzgerald et al. are the first to report a frequency of AT heterozygotes in a population based on screening of the ATM gene (14). Though the total number screened is relatively small (200 individuals), and the PTT assay utilized may underestimate the number of AT mutations, the reported AT carrier frequency of 1% agrees with estimates from previous epidemiological studies (11, 17, 20) and supports estimates that 4-18% of all breast cancer patients may be AT heterozygotes. ATM heterozygosity, therefore, potentially accounts for a greater proportion of breast cancer cases than BRCA1, which accounts for an estimated 3 % of all U.S. breast cancer cases diagnosed by 80 years (10). In our study, 11 of 142 early onset breast cancer cases diagnosed in western Washington have single amino acid variants in ATM. Missense mutations can disrupt ATM functions and cause disease. Further analysis will be necessary to confirm whether a single ATM missense mutant confers susceptibility to breast cancer, but the results up to now are suggestive, based on position of the putative mutations within highly conserved regions, or previous sighting of the mutation in AT patients. Because of the relatively high frequency of AT carriers in the general population, it is important to identify them, as they may be predisposed to breast cancer and hypersensitive to standard therapeutic doses of radiation.

Bibliography

1. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, et al. (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266:66-71.

2. Claus EB, Risch NJ, Thompson WD (1991) Genetic analysis of breast cancer in the cancer and steroid hormone study. Am J Hum Genet 48:232-242.

3. Serova OM, Mazoyer S, Puget N, Dubois V, Tonin P, Shugart YY, Goldgar D, Narod SA, Lynch HT, Lenoir GM (1997) Mutations in BRCA1 and BRCA2 in breast cancer families: are there more breast cancer-susceptibility genes? Am J Hum Genet 60:486-495.

4. Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, Bishop DT, et al. (1998) Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. Am J Hum Genet 62:676-689.

5. Schubert EL, Lee MK, Mefford HC, Argonza RH, Morrow JE, Hull J, Dann JL, King M-C (1997) BRCA2 in American families with four or more cases of breast or ovarian cancer: recurrent and novel mutations, variable expression, penetrance, and the possibility of families whose cancer is not attributable to BRCA1 or BRCA2. Am J Hum Genet 60:1031-1040.

6. Vehmanen P, Friedman LS, Eerola H, McClure M, Ward B, Sarantaus L, Kainu T, et al. (1997) Low proportion of BRCA1 and BRCA2 mutations in Finnish breast cancer families: evidence for additional susceptibility genes. Hum Mol Genet 6(13):2309-2315.

7. Langston AA, Malone KE, Thompson JD, Daling JR, Ostrander EA (1996) BRCA1 mutations in a population-based sample of young women with breast cancer. N Engl J Med 334(3):137-142.

8. FitzGerald MG, MacDonald DJ, Krainer M, Hoover I, O'Neil E, Unsal H, Silva-Arrieto S, et al. (1996) Germ-line BRCA1 mutations in Jewish and non-Jewish women with early-onset breast cancer. N Engl J Med 334:143-149.

9. Struewing JP, Hartge P, Wacholder S, Baker SM, Berlin M, McAdams M, Timmerman MM, et al. (1997) The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. N Engl J Med 336:1401-1408.

10. Whittemore AS, Gong G, Itnyre J (1997) Prevalence and contribution of BRCA1 mutations in breast and ovarian cancer: results from three U.S.

population-based case-control studies of ovarian cancer. Am J Hum Genet 60:496-504.

11. Swift M, Reitnauer PJ, Morell D, Chase CL (1987) Breast and other cancers in families with Ataxia-Telangiectasia. N Eng J Med 316(21):1289-1294.

12. Gatti RA (1991) Localizing the Genes for Ataxia-Telangiectasia: A Human Model for Inherited Cancer Susceptibility. Adv Cancer Res 56:77-104.

13. Morell D, Chase CL, Swift M (1990) Cancer in 44 families with Ataxia-Telangiectasia. Cancer Genet Cytogenet 50:119-123.

14. Fitzgerald MG, Bean JM, Hedge SR, Unsal H, MacDonald DJ, Harkin DP, Finkelstein DM, Isselbacher KJ, Haber DJ (1997) Heterozygous ATM mutations do not contribute to early onset of breast cancer. Nature Genet 15:307-310.

15. Paterson MC, Anderson AK, Smith BP, and Smith PJ (1979) Enhanced radiosensitivity of cultured fibroblasts from ataxia-telangiectasia heterozygotes manifested by defective colony-forming ability and reduced DNA repair replication after hypoxic gamma irradiation. Cancer Res. 39:

16. Young BR, Painter RB (1989) Radioresistant DNA synthesis and human genetic diseases. Hum. Genet. 82:113-117.

17. Swift M, Morrell D, Massey RB, Chase CL (1991) Incidence of cancer in 161 families affected by Ataxia-Telangiectasia. N. Eng. J. Med. 325(26):1831-1836.

18. Pippard EC, Hall AJ, Barker DJP, Bridges BA (1988) Cancer in Homozygotes and heterozygotes of Ataxia-Telangiectasia and Xeroderma Pigmentosum in Britain. Cancer Res. 48:2929-2932.

19. Borresen A-L, Andersen TI, Tretli S, Helberg A, Moller P (1990) Breast Cancer and other cancers in Norwegian families with ataxia-telangiectasia. Genes, Chrom. & Canc. 2:339-340.

20. Easton DF (1994) Cancer risks in A-T heterozygotes. Int J Radiat Biol 66:S177-S182.

21. Athma P, Rappaport R, Swift M (1996) Molecular genotyping shows that ataxia-telangiectasia heterozygotes are predisposed to breast cancer. Cancer Genet Cytogenet 92:130-134.

22. Savitsky K, Bar-Shiva A, Gilad S, Rotman G, Ziv Y, et al. (1995) A single Ataxia Telangiectasia gene with a product similar to PI-3 kinase. Science 268:1749-1753.

23. Savitsky K, Sfez S, Tagle DA, Ziv Y, Sartiel A, Collins FS, Shiloh Y, Rotman G (1995b) The complete sequence of the coding region of the ATM gene reveals similarity to cell cycle regulators in different species. Hum Mol Genet 4(11):2025-2032.

24. Marx J (1997) Possible function found for breast cancer genes. Science 276:531-532.

25. Platzer M, Rotman G, Bauer D, Uziel T, Savitsky K, Bar-Shira A, Gilad S, Shiloh Y, Rosenthal A (1997) Ataxia-telangiectasia locus: sequence analysis of 184 kb of human genomic DNA containing the entire ATM gene. Genome Res 7:592-605.

26. Uziel T, Savitsky K, Platzer M, Ziv Y, Helblitz T, Nehls M, Boehm T, Rosenthal A, Shiloh Y, Rotman G (1996) Genomic organization of the ATM gene. Genomics 33:317-320.

27. Concannon P, Gatti RA (1997) Diversity of ATM gene mutations detected in patients with Ataxia-telangiectasia. Hum Mut 10:100-107.

28. Gilad S, Khosravi R, Shkedy D, Uziel T, Ziv Y, Savitsky K, Rotman G, et al. (1996) Predominance of null mutations in ataxia-telangiectasia. Hum Mol Genet 5 (4):433-439.

29. Vorechovsky I, Luo L, Dyer JS, Catovsky D, Amlot PL, Yaxley JC, Foroni L, et al. (1997) Clustering of missense mutations in the ataxia-telangiectasia gene in a sporadic T-cell leukemia. Nature Genetics 17:96-99

30. StilgenBauer S, Schaffner C, Litterst A, Leibisch P, Gilad S, Bar-Shira A, James MR, et al. (1997) Biallelic mutations in the ATM gene in T-prolymphocytic leukemia. Nature Medicine 3:115-1159.

31. Stankovic T, Kidd AMJ, Sutcliffe A, McGuire GM, Robinson P, Weber P, Bedenham T et al. (1998) ATM mutations and phenotypes in Ataxia-telangiectasia families in the British Isles: expression of mutant ATM and the risk of leukemia, lymphoma, and breast cancer. Am J Hum Genet 62:334-345.

32. Vorechovsky I, Rasio D, Luo L, Monaco C, Hammarstrom L, Webster ADB, Zaloudik J, et al. (1996) The ATM gene and susceptibility to breast cancer:

analysis of 38 breast tumors reveals no evidence for mutation. Cancer Res 56:2726-2732.

33. Vorechovsky I, Luo L, Lindblom A, Negrini M, Webster ADB, Croce CM, Hammarstrom L (1996) ATM mutations in cancer families. Cancer Res 56:4130-4133

34. Orita M, Suzuki Y, Sekiya T, Hayashi K (1989) Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 5:874-879.

35. Beaudet AL, Tsui L-C (1993) A suggested nomenclature for designating mutations. Hum Mutat 98:245-248.

36. Shafman T, Khanna KK, Kedar P, Spring K, Kozlov S, Yen T, Hobson K, Gatei M, Zhang N, Watters D, Egerton M, Shiloh Y, Kharbanda S, Kufe D, Lavin MF (1997) Interaction between ATM protein and c-Abl in response to DNA damage. Nature 387:520-523.

37. Baskaran R, Wood LD, Whitaker LL, Canman CE, Morgan SE, Xu Y, Barlow C, Baltimore D, Wynshaw-Boris A, Kastan MB, Wang JYJ (1997) Ataxia telangiectasia mutant protein activates c-Abl tyrosine kinase in response to ionizing radiation. Nature 387:516-519.

38. Izatt L, Hodgson SV, Solomon E (1997) A study of the frequency of germline ataxia-telangiectasia mutated mutations in young breast cancer patients. Abstracts of Ataxia-telangiectasia and ATM: Functional, Genetic and Clinical Ramifications-Baltimore MD.

39. Cooper TA and Mattox W (1997) Gene Regulation '97—The regulation of splice-site selection, and its role in human disease. Am J Hum Genet 61:259-266.

40. Bishop DT and Hopper J (1997) AT-tributable risks? Nature Genet 15:226.

41. Tokunaga M, Land CE, Yamamoto T et al. (1987) Incidence of female breast cancer among atomic bomb survivors, Hiroshima and Nagasaki, 1950-1980. Radiat. Res. 112:243-272.

42. Boice JD, Rosenstein M, Trout ED (1978) Examination of breast doses and breast cancer risk associated with repeated fluoroscopic chest examinations of women with tuberculosis. Radiat. Res. 73:373-390

43. Scully R, Chen J, Plug A, Xiao Y, Weaver D, Feunteun J, Ashley T, Livingston D (1997) Association of BRCA1 with Rad51 in mitotic and meiotic cells. Cell 88:265-275.

44. Plug A, Peters AHFM, Xu Y, Keegan KS, Hoekstra MF, Baltimore D, deBoer P, Ashley T (1997) ATM and RPA in meiotic chromosome synapsis and recombination. Nature genet 17:457-461.

45. Sharan SK, Morimatsu M, Albrecht U, Lim D-S, Regel E, Dinh C, Sands A, et al. (1997) Embryonic lethality and radiation hypersensitivity mediated by Rad51 in mice lacking BRCA2. Nature 386:804-810.

46. Barlow C, Hirotsune S, Paylor R, Liyanage M, Eckhaus M, Collins F, Shiloh Y, et al. (1996) Atm-deficient mice: a paradigm of ataxia telangiectasia. Cell 86:159-171.

47. Xu Y, Ashley T, Brainerd EE, Bronson RT, Meyn S, Baltimore D (1996) Targeted disruption of ATM leads to growth retardation, chromosomal fragmentation during meiosis, immune defects, and thymic lymphoma. Genes & Dev 10:2411-2422.

48. Elson A, Wang Y, Daugherty CJ, Morton CC, Zhou F, Campos-Torres J, Leder P. (1996) Pleiotropic defects in ataxia-telangiectasia protein-deficient mice. Proc Natl Acad Sci 93:13084-13089.

49. Hakem R, de la Pompa JL, Sirard C, Mo R, Woo M, Hakem A, Wakeham A, et al. (1996) The tumor suppressor gene BRCA1 is required for embryonic cellular proliferation in the mouse. Cell 85:1009-1023.

50. Connor F, Bertwistle D, Mee PJ, Ross GM, Swift S, Grigorieva E, Tybulewica VLJ, Ashworth A (1997) Tumorigenesis and a DNA repair defect in mice with a truncating BRCA2 mutation. Nature genet 17:423-30.

Table 1. ATM primers used to amplify genomic DNA

Exon	Primer sequence 5' to 3'	Product size, bp	Anneal temp. °C
Exon 4F	CCTCTTTCTCTCTATATATGC	160	58
Exon 4R	AATAATGGGTTACTAATCACA		58
Exon 5.1F	GATTAGTAACCCATTATTATTC	210	56
Exon 5R	CAACAGAAATAAATATGAAAGAG		58
Exon 6.1F	GATGGCATGAACAGCTTTTG	280	58
Exon 6R	CTCACGCGACAGTAATCTG		58
Exon 7F	TAGTTGCCATTCCAAGTGTC	288	58
Exon 7R	TGAAGTTTCATTTCATGAGG		58
Exon 8F	TTTTTCTGTATGGGATTATGGA	327	58
Exon 8R	CATGGTCTTGCAAGATC		58
Exon 9F	CCCCCTGTTATACCCAGTT	318	58
Exon 9R	TGAAGAAGCAAATTCAAAACAG		58
Exon 10F	TTTGTGGGGAGCTAGCAGTG	262	58
Exon 10.1R	TCTAAATGTGACATGACCTAC		58
Exon 11.1F	GGCTCAAAAAAAAAAAAAAG	265	58
Exon 11R	ACAAGAGATTAAAATGACACT		58
Exon 12F	GTTTGTTAATGTGATGGAATA	467	58
Exon 12R	GTGTGTTTATCTGTAAGTCAG		58
Exon 13F	ATAAAGTCTTTGCCCCTCCA	320	58
Exon 13R	AAATAAGTGGAGAGAGCCTG		58
Exon 14F	GGCTTTTGGTCTTCTAAGTG	192	58
Exon 14R	ATCTTTGTAATTAAAGCTATAGC		58
Exon 15F	GTAGTCTTTGAATGATGTAGA	377	56
Exon 15R	CTATTTCTCCTTCCTAACAGT		58
Exon 16.2F	GTTCTTACAAAAGATAGAGTATAC	329	62
Exon 16.2R	TTCACAGGAATACATTTCATTC		56
Exon 17.2F	GTCCAAGATCAAAGTACACTG	314	60
Exon 17.2R	GTGACAGAGAAAGATCCTATC		60
Exon 18F	ATATTGGCCCTAATAGTAAAC	292	54
Exon 18.2R	CCTTATTTACAAAGATATTTCAAC		60
Exon 19F	AATTGCTGAGATTACAGATGT	352	56
Exon 19.1R	GCCTCTTATACTGCCAAATCA		60
Exon 20.2F	TATATATGGCTGTTGTGCCC	314	58
Exon 20R	TACATTTAGTCAGCAACATCA		56
Exon 21F	CCGGCCTATGTTTATATACTT	225	58
Exon 21R	TTAACAGAACACATCAGTTAT		54
Exon 22.2F	AACTGATGTGTTCTGTTAAGC	274	58
Exon 22R	CTTGCATTCGTATCCACAGAT		60
Exon 23F	TTAGCACAGAAAGACATATTG	259	56
Exon 23R	AATTACTCATTAACAAACAAA		50

Exon 24F	GCAGTCTTTGTTTGTTAATGA	274	56
Exon 24R	CTATGTAAGACATTCTACTGC		58
Exon 25F	GTTTGTTTGCTTGCTTGTTT*	203	54
Exon 25R	ATTTATGGGATATTCATAGC*		52
Exon 26F	TGGAGTTCAGTTGGGATTTTA*	304	58
Exon 26R	TTCACAGTGACCTAAGGAAGC*		62
Exon 27F	GTTGTTTCTAGGTCCTACTCT	333	60
Exon 27R	GACTTGCTAAGTATTGTTAAC		56
Exon 28F	TGATACTTTAATGCTGATGGT	409	56
Exon 28R	GGTTATATCTCATATCATTCA		54
Exon 29F	TCCTCTTAGTCTACAGGTTG	257	58
Exon 29R	GACATTGAAGGTGTCAACCA		58
Exon 30F	TGGAAGTTCACTGGTCTATG	283	58
Exon 30R	TACTTTTCCTCTTTAAGATGTAT		58
Exon 31F	TTTATTGTAGCCGAGTATCTAA	318	58
Exon 31R	AAACAGGAAGAACAGGATAGA		58
Exon 32F	TGCTGAACCAAAGGACTTCT	334	58
Exon 32R	CACTCAAATCCTTCTAACAATA		58
Exon 33F	CAGTAAGTTTTGTTGGCTTAC	315	58
Exon 33R	CTGCTAGAGCATTACAGATTT		58
Exon 34F	TGTCTATAAATGGCACTTAACT	311	58
Exon 34R	CCAAGAGCAAGACTTTGCAAA		58
Exon 35F	TAGAAGTTTTCTAGTCAGATAAT	255	58
Exon 35R	AATCTGTCCTATATGTGATCC		58
Exon 36F	CTTGAAGTACAGAAAAACAGC	336	58
Exon 36R	GTATCATTCTCCATGAATGTC		58
Exon 37F	TGGAGGTTAACATTCATCAAG	287	58
Exon 37R	ATTTAACAGTCATGACCCACA		58
Exon 38F	GGAAAGGTACAATGATTTCCA	312	58
Exon 38R	ATGTGCAGTATCACAGCACT		58
Exon 39F	GTATGTTGAGTTTATGGCAGA	376	58
Exon 39R	ATCCATCTTTCTCTAGAACTG		58
Exon 40F	ACCAGAACCTTATAGCATAGT	247	58
Exon 40R	TTCAGCCGATAGTTAACAAGT		58
Exon 41F	TAAGCAGTCACTACCATTGTA	314	58
Exon 41R	ATACCCTTATTGAGACAATGC		58
Exon 42F	GTATATGTATTCAGGAGCTTC	238	58
Exon 42R	ATGGCATCTGTACAGTGTCT		58
Exon 43F	CAGAACTGTATTTCAGAATCAT	387	58
Exon 43R	ACATAACTCCTTCATAAACAGT		58
Exon 44F	CCAAAGCTATTTTCACAATCTT	262	58
Exon 44R	TACTGAAATAACCTCAGCACT		58
Exon 45F	CTCTGGTTTTCTGTTGATATC	236	58
Exon 45R	CCCCATGAAGAATCAAGTC		58

Exon 46F	TTTATACATGTATATCTTAGGGTTCTG	220	58
Exon 46R	TTCAGAAAAGAAGCCATGACA		58
Exon 47F	TATTTCCCTGAAAACCTCTTC	233	58
Exon 47R	CACTATTGGTAACAGAAAAGC		58
Exon 48F	TCATTTCTCTTGCTTACATGAA	314	58
Exon 48R	AAAGGAAAGTCAAGAGGTAAG		58
Exon 49F	ATGGTAGTTGCTGCTTTCATT	365	58
Exon 49R	TTACTAATTTCAAGGCTCTAATA		58
Exon 50F	AGTTGGGTACAGTCATGGTA	230	58
Exon 50R	GAAAAGATGAAGCATATTCATG		58
Exon 51F	TTTGAGTGATTCTTTAGATGTAT	352	58
Exon 51R	AACAACTCACTCAGTTAACTG		58
Exon 52F	TGTGTGATTTTGTAGTTCTGTT	340	58
Exon 52R	ACATCAAGGGGCTTATGTCT		58
Exon 53F	ACTTACTTGCTTAGATGTGAG	282	58
Exon 53R	CCATTTCTTAGAGGGAATGG		58
Exon 54F	CACTGCAGTATCTAGACAGT	322	58
Exon 54R	CTAGGAAAGACTGAATATCAC		58
Exon 55F	AATGTTGGGTAGTTCCTTATG	308	58
Exon 55R	GCTTTTGGATTACGTTTGTGA		58
Exon 56F	TGACTATTCCTGCTTGACCT	253	58
Exon 56R	TTTCACCAATTTTGACCTACAT		58
Exon 57F	TAACCACTATCACATCGTCAT	385	58
Exon 57R	CTTCCTCATTTGTAAGTATTCA		58
Exon 58F	CCTTTGCTATTCTCAGATGACTCTGT	290	58
Exon 58R	GCATTATGAATATGGGCATGA		58
Exon 59F	GATCATCAAATGCTCTTTAATG	286	58
Exon 59R	TATCTGACAGCTGTCAGCTT		58
Exon 60 F	GTGTATATTAGTTTAATTGAACAC	279	58
Exon 60R	AACCTGCCAAACAACAAAGTG		58
Exon 61F	TAGAAAGAGATGGAATCAGTG	317	58
Exon 61R	ATCTTGGTAGGCAAACAACAT		58
Exon 62F	AAAGTTCACATTCTAACTGGAA	272	58
Exon 62R	ATTACAGGTGCAAAGAACCAT		58
Exon 63F	GATAAAGATACGTTGTTGACAACATTGG	199	58
Exon 63R	GTGACTTCCTGATGAGATACACAG		58
Exon 64F	CTGGTTCTACTGTTTCTAAGT	298	58
Exon 64R	GTTTCAGTGAGGTGAACAGT		58
Exon 65F	TCCTGTTGTCAGTTTTTCAGA	354	58
Exon 65R	ACTTAAAGTATGTTGGCAGGT		58

* Exon 25 and 26 primer sequences are taken from Vorechovsky et al.(24).

Table 2. Putative ATM Missense Mutations and Rare Variants

Exon[a]	Nucleotide change[b,c]	Codon change[d]	Frequency in breast cancer cases[e]	Frequency in controls[e]
A. Putative ATM Missense Mutations				
8	609C>A	203D>E	1/284	0/226
15	2119T>C	707S>P	6/284	1/284
30	4158C>T	1383S>L	1/284	0/222
54	IVS54+8A>G	--	1/284	0/226
62	8734A>G	2912R>G	1/284	0/224
65	9031A>G	3011M>V	1/284	0/226
B. ATM rare variants				
7	370A>G	124I>V	1/284	0/218
7	IVS7+18T>C	--	1/284	0218
12	1541G>A	514G>E	1/284	1/224
16	IVS16+22A>C, IVS16+34A>C	--	2/284	0/164
19	2572T>C	858F>L	3/284	6/215
20	IVS19-17G>T	--	0/284	1/220
20	2805G>C	935T>T	1/284	0/220
25	IVS25+32delCAT	--	1/284	0/218
32	4742C>T	1526P>P	20/204	7/164
36	5071A>C	1691S>R	2/284	0/228
39	5558A>T	1853D>V	2/284	3/366
41	5793T>C	1931A>A	1/284	1/162
43	6088A>G	2030I>V	1/284	1/226
49	6919C>T	2307L>F	1/284	0/164
50	6988C>G	2330L>V	1/284	0/226
62	IVS62+8A>C	--	4/284	4/224

[a]Exons are numbered in accordance with Uziel et al. (19).

[b]Nucleotide numbering is based on the sequence reported by Savitsky et al. (17) (GenBank accession number U33841) in which position 1 is the first nucleotide of the start codon, and putative mutations and variants are named in accordance with the convention of Beaudet and Tsui (25), modified by Antonarakis (http://ariel.ucs.unimelb.edu.au:80/~cotton/antonara.htm)

[c]IVS refers to introns and nucleotides therein are numbered such that the splice acceptor AG is numbered -2,-1 and the splice donor GT is numbered +1,+2.

[d]The first methionine in the open reading frame is at position 1.

[e]Number of occurrences of the less frequent allele/total number of chromosomes screened.

Table 3. ATM exons compatible with multiplex SSCP analysis.

ATM Exons grouped for quadruplex or triplex analysis:

5, 10, 38, 65

8, 29, 61, 44

19,26, 11, 6

51, 31, 55

36, 18, 42

33, 17, 56, 50*

49, 48, 30, 47

32, 35, 40, 4

14, 45, 52

24, 27, 53, 63

12, 15, 13, 63

28, 59, 58*, 25

57, 54, 21

41, 22, 46

7, 9, 64*

*PCR product was weak in the multiplex reaction.

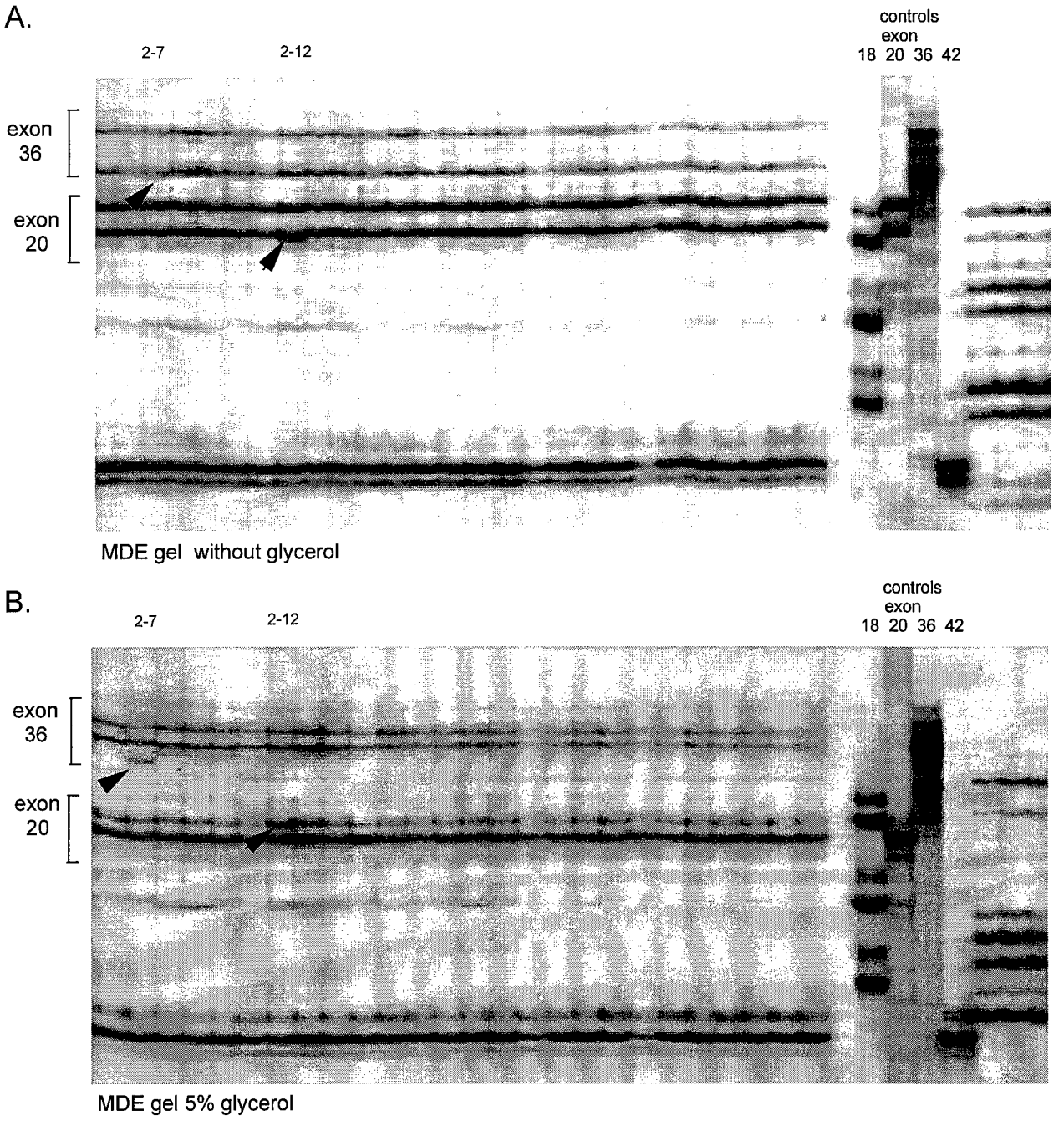

Figure 1. SSCP analysis of a panel of early-onset breast cancer cases for ATM variants: comparison between A., MDE gel without glycerol and B., MDE with 5%glycerol. Arrows point to the variant band in exon 36 for patient 2-7 and the variant band in exon 20 for patient 2-12. The exon 36 variant is more readily detected in the 5% glycerol gel, while the exon 20 variant is more readily detected in the non-glycerol gel. Exon 18 overlaps with exon 20, but does not amplify well in the multiplex reaction. Exon 18 contains a common polymorphism that is represented by a slight shift in the lower band of the SSCP analysis.

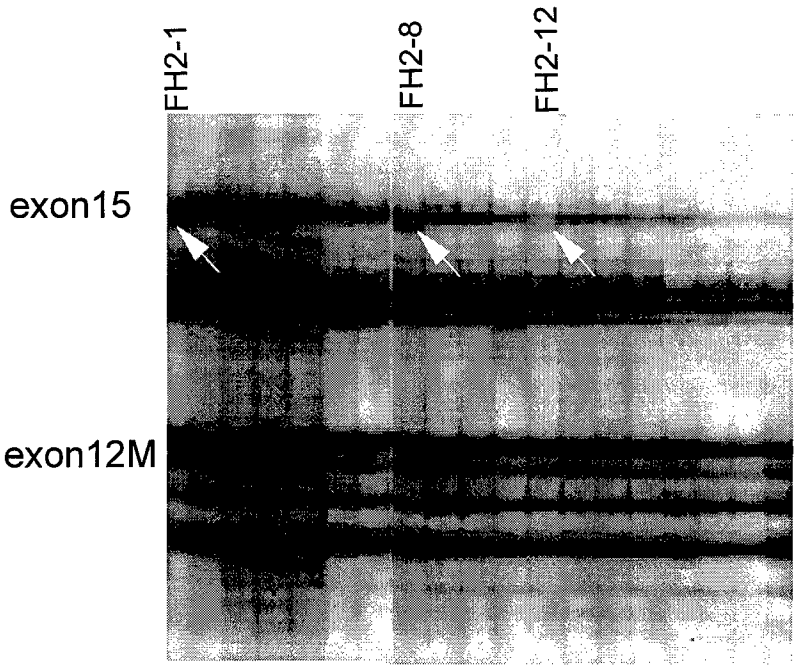

Figure 2. ATM exon 15 mutation 2119T>C, Ser707Pro, in early-onset breast cancer cases. SSCP analysis on MDE with 5% glycerol. Arrows indicate the variant bands corresponding to ATM 2119 C alleles. Exon 12M, which shows no variants on this panel, refers to ATM 1338-1607 and flanking nucleotides.

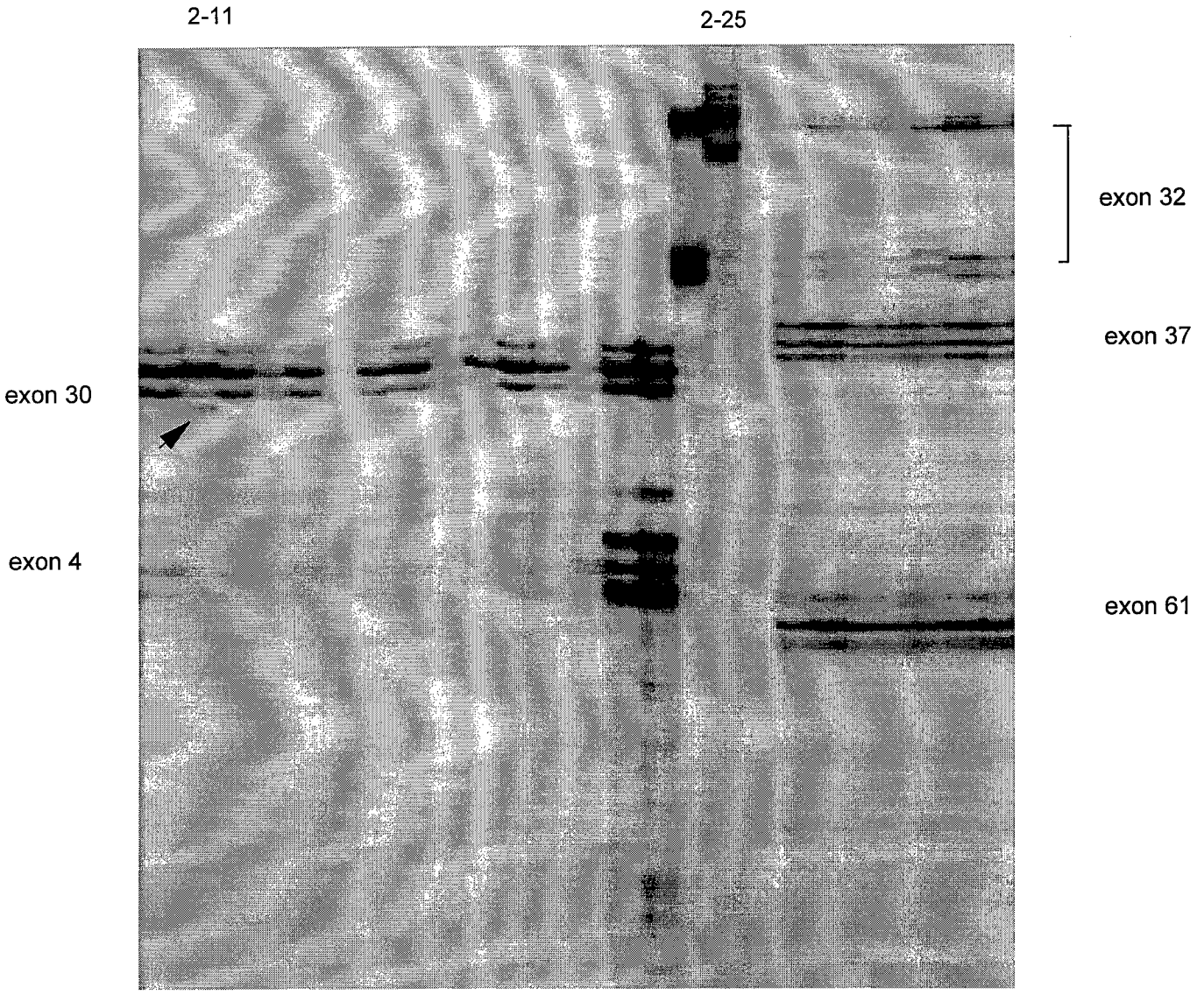

Figure 3. SSCP Analysis of a panel of early-onset breast cancer cases for ATM variants: putative missense mutation in exon 30 for patient 2-11. The arrow points to the exon 30 variant. The SSCP for exon 32 shows the presence of a common polymorphism. Exon 4 also contains a common polymorphism.

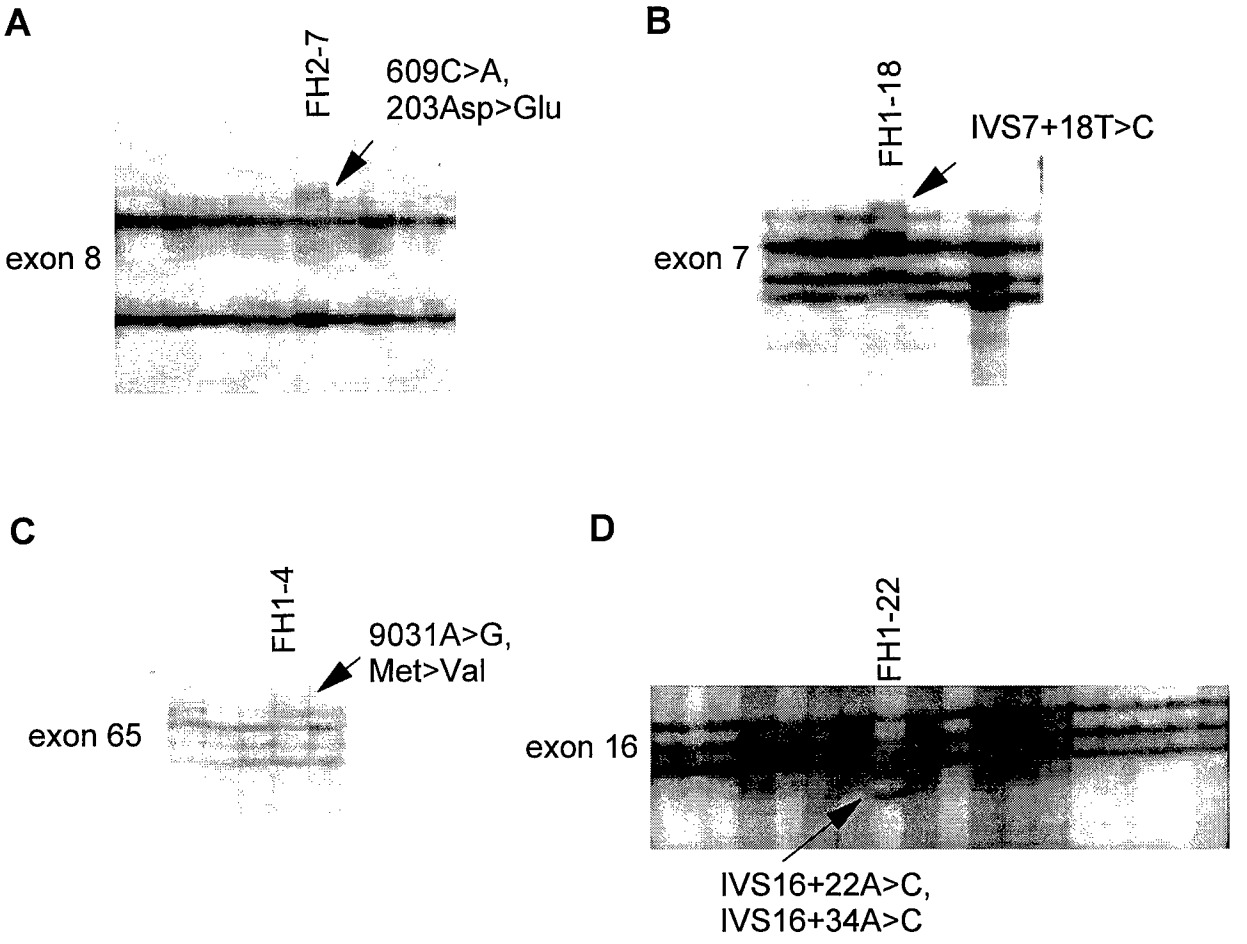

Figure 4. Novel ATM variants in early-onset breast cancer cases. SSCP analyes on MDE matrices with 5% glycerol detects single base substitutions in the genomic DNA of breast cancer cases in 4A, B, and C, while 4D shows the band shift caused by a 2 base substitution. None of these variants appeared in controls.

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 19 Jan 01

MEMORANDUM FOR Administrator, Defense Technical Information Center, ATTN: DTIC-OCA, 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for Grant DAMD17-96-1-6160. Request the limited distribution statement for Accession Document Numbers ADB232741, ADB241196, ADB255697 be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by email at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

PHYLLIS M. RINEHART
Deputy Chief of Staff for
Information Management